Fitness Effects of Horizontal Gene Transfer from *Wolbachia* to *Drosophila*
By Olga M. Better

~~The relationship between infectious diseases and hosts have been a long and continuous battle than is constantly evolving. Having a better understanding of how hosts and pathogens interact will help us understand this constant evolution. One approach to studying pathogens is to use lab models like *Drosophila* to study the complicated relationship between parasites and organisms. *Wolbachia* is a parasite that invades *Drosophila* and causes feminization of the offspring. *Wolbachia* feminizes *Drosophila* offspring, manipulating cellular processes to favor its own transmission. We expect this phenotype to impact their overall fitness, either in a positive or negative way. The propose of my research is to identify mutations (novel genes) in *Drosophila* that have been infected with *Wolbachia*, figure out which genes are affected, and to see if these mutations are ever beneficial to the fitness of *Drosophila*.~~

Aims

1. a) Identify DNA that has been transferred from *Wolbachia* to *Drosophila* by pulling out sequenced read-pairs that have one read on the *Wolbachia* chromosome, and one read one the Drosophila's chromosome. Genuine mutations should be supported by multiple pairs of abnormally mapping read-pairs.
   b) Characterize new genes formed via HGT via RT-PCR, RNA Seq, and fluorescent in situ hybridization. Looking at the gene expression patterns of newly transferred *Wolbachia* DNA to identify cases of new gene formation.

2. Looking at a minimum 20 strain subset of our 150 different Drosophila strains to see the why some *Drosophila* thrive and others do not via phenotypic assays on stressed *Drosophila* (temp shock, starvation, and chemical mutagens).

3. Explore evolutionary dynamics of these novel genetic mutations. By looking at the phylogeny of HGT segments in *Drosophila*.

Approach:
The Rogers lab is generating population genomic sequencing panels for 100 strains of *Drosophila yakuba*, a sister species of the model *D. melanogaster*. Recent work has shown that natural populations of *D. yakuba* and related species harbor *Wolbachia* (Cooper et al 2016). One impact *Wolbachia* can have on their *Drosophila* hosts, is that they can transfer DNA to the nucleus via horizontal gene transfer. I will be looking for mutations that have inserted *Wolbachia* DNA into all 4 major *Drosophila* chromosomes. I will pull out sequenced read-pairs that have one read the *Wolbachia* chromosome, and one read on the *D. yakuba* chromosome. I will then cluster the reads to find where multiple reads match the same place in the *Wolbachia* genome and the same place in the *D. yakuba* genome. Genuine mutations will be identified as cases where three or more abnormally mapping read-pairs suggest transfer of DNA from *Wolbachia* to the nucleus.

Once we have identified mutations, we will focus on the fitness of *Drosophila* and the effect of the mutation. I will look at gene expression experiments via RT-PCR, RNA-Seq, and *in situ* hybridization to identify tissues where new genes may have formed because of HGT. I will then look for genetic advantages in some *Drosophila* stains vs others. I can then stress the *Drosophila* via temp shock, starvation, and chemical mutagens to see whether strains with new, *Wolbachia*-derived genes are more likely to be resistant to stresses. Conversely, HGT from *Wolbachia* may disrupt genes, resulting in key elements being lost. Such gene disruption may cause the fitness of *Drosophila* to decline.

Finally, I can use the phylogeny of HGT segments in the 100 stains *Drosophila yakuba* to track evolutionary dynamics of these mutations over time. Tracking HGT events across the phylogeny to see if *Wolbachia* DNA fragments are in *Drosophila* offspring, and counting total offspring number with these mutations. If we continue to see these reads in all the future generations we can confirm HGT, and see if mutations persist every generation or it if it's a one-off event. Stable incorporation of *Wolbachia* DNA into the nucleus may indicate mutations that happen to offer a fitness benefit to the hosts. This same analysis can be expanded easily to other species of *Drosophila*, including *D. melanogaster* and *D. simulans* public population genomic panels

Broader Impacts:
Novel genetic mutations can unlock the reason why we see different strains of *Drosophila* with *Wolbachia* thriving but others slowly decline in numbers. These novel mutations can help us understand how and why *Wolbachia* feminizes *Drosophila* offspring thus effecting their overall fitness, either in a positive or negative way. Characterizing these novel genes will give us insights on the relationship between *Wolbachia* and *Drosophila*. A deeper understanding of co-dependences between *Wolbachia* and *Drosophila* could determine if these novel mutated genes are being created via horizontal gene transfer. These answered questions will help us have a better understand horizontal gene transfer and how this impact pathogen success.

Understanding pathogen evolution will benefit humans in many different ways. For example, a number of scientists are trying to produce sterile mosquitos with the help of *Wolbachia* to reduce the number of malaria. Male mosquitoes are bred and infected with *Wolbachia*, then they are let go into area with high number of malaria cases. There the infected males and uninfected females mate and the resulting eggs that the females lay will not hatch due to cytoplasmic incompatibility. A sterile mosquito would cause mosquito populations to crash in high malaria regions. Thus, lowing the spread of malaria around the world.

Olga Better proposed a graduate research project on the ways pathogens influence evolutionary fitness in *Drosophila.* Dr. Rogers suggested HGT between *Wolbachia* and *Drosophila* as the best match between their shared interests. Olga Better designed follow up experiments with minor changes suggested by Dr. Rogers. Olga Better drafted the proposal with editing from Dr. Rogers.

Cooper, B.S., Ginsberg, P.S., Turelli, M. and Matute, D.R., 2017. *Wolbachia* in the *Drosophila yakuba* complex: pervasive frequency variation and weak cytoplasmic incompatibility, but no apparent effect on reproductive isolation. *Genetics*, *205*(1), pp.333-351.